Buyer Beware - A guide to selecting and purchasing antibodies

Hi all,
I realize that the video title is a bit of clickbait, but I still think it applies pretty well to an issue I wanted to talk about: trying not to buy the wrong antibody. This is a problem that a lot of researchers encounter, especially if they haven't had to pick their antibodies from a vendor before. In particular, people may end up ordering an antibody that looks good for their purpose, but ultimately fails to stain the target. Further, primary antibodies - those that bind directly to the target - are typically quite expensive, running between $200 to $600. So, if you end up buying the wrong one and are unable to return it, that's an expensive mistake. Let's talk about some of these antibody selection issues. I'll also pose some questions for you to answer. When I ask each question, I recommend pausing the video to try to figure out the answer on your own before I discuss my answer. A few disclaimers before I get started. First, most of these figures were not created by me, so I have put source links next to the images. Second, although I will be showing pages for specific antibodies, I do not endorse nor discourage purchase of any of the products shown. I am not receiving support from any of these vendors. With that out of the way, let's get into it.

1. How do antibodies made by a vendor bind to a very specific target?
    a. Generally, vendors isolate a specific amino acid sequence from a larger protein. So, if they are making an antibody that can selectively target a dopamine 1 receptor, they won't use the whole intact dopamine receptor. Instead, they may do something like cleave off the last twenty amino acids of the c-terminus of the receptor. This twenty amino acid strand is then exposed to immune cells to make the antibody against that. If the vendor is touting that their antibody is highly specific, such that it does not bind to similar proteins, then they've likely chosen a sequence that has little similarity to most other proteins, or at least the proteins found in the organ of interest. An example of this might be that a vendor's antibody is able to target dopamine 1 receptors yet it does not target dopamine 2 receptors.
2. On a related note, how are antibodies specific to the protein of one species and yet not target the same protein in a different species?
    a. It turns out that even if one species has the same *general* protein structure for a specific target as another species, the small differences in the sequences between species are enough to prevent the antibody from binding to one of those species. For example, if an antibody is meant to target the human dopamine 1 receptor in humans, it isn't necessarily a guarantee that it will bind to the same dopamine 1 receptor in mice. The sequence the vendor isolates may be just different enough between human and mouse such that the antibody will not bind to a mouse dopamine 1 receptor.
    [diagram would be helpful]
    b. You might be wondering, "how do I figure out if an antibody might work for a species the vendor doesn't specify?" You might be able to determine that by looking it up on NIH's BLASTp site. If the vendor gives you the target amino acid sequence, you can put it into BLASTp and have it search for a list of matches.

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome

c. However, this webpage requires either a specific sequence or an identification number (in this case, ascension number). I recommend looking up the protein via some sort of database. I used the wikipedia entry for dopamine receptor D1.
https://en.wikipedia.org/wiki/Dopamine_receptor_D1
I then copied one of the RefSeq (protein) identifiers. NP_000785
[demonstrate BLASTp search]
If the sequence matches exactly between species, then it should work! If it there are a few mismatches between species, then it is very much up to chance about the antibody working or not. And, if the sequences are mostly non-matching, then it's very likely that it won't work on a species that the vendor did not test.

d. Let's do another example, this time with a much more narrow target. A vendor tells me that their antibody targets "an immunogen that is a synthetic peptide within Human NMDAR1 amino acids 849-913".
https://www.abcam.com/products/primary-antibodies/nmdar1-antibody-ab52177.html
Let's say you wanted to know if it will bind to NMDA receptors in a rat brain. This time, I will use an alternate database, as the vendor has supplied a UniProt number and this refers the www.uniprot.org database. We can run BLAST on that website. When it is ready, we can select the homo sapiens check box, then type "rattus" into the search tool box. We'll then check the rattus norvegicus result, then click on Align. We'll be brought to the Align page with the sequences already entered, so we just run it. When it is finished, we see the full sequences of the NMDA receptor aligned side-by-side, with highlighting indicating the differences. We can browse around the area of amino acids 849 through 913, and we see that there are no differences there. So, in theory, the antibody could work on rat brain tissue. However, nothing is an absolute guarantee, and if you are set on purchasing that specific antibody, you should probably contact the vendor's customer service to see what they think about the sequence homology.

3. Why does it matter what "host" the antibody comes from?
    a. The host of the antibody helps you decide what secondary antibody you will use. This is very important when you have a limited array of secondary antibodies in your lab's collection. For example, if you only have donkey anti-mouse antibodies as your secondaries, then you should only purchase primary antibodies that come from the host species of mouse.
4. Do you know the difference between monoclonal vs polyclonal antibodies?
    a. Polyclonal antibodies are derived from taking a target sequence of amino acid and injecting it into an animal. The immune fraction of that animal's blood is isolated, and from that the antibodies are isolated further into the specific antibodies directed at the target. The name polyclonal indicates that the antibodies come from different immune cells, and thus these immune cells create antibodies that attach to different parts of the target sequence. This diversity of antibodies increases the probability that the product will bind to the target, but it also increases the possibility of non-specific antibody binding. (As a side note, the result of non-specific antibody binding is increased background staining.
    b. Monoclonal antibodies are derived from modified immune cells in vitro. The cells are genetically the same, so the antibodies they produce also attach to the same exact part of the target sequence. This identical nature of all the antibody molecules ensures that

all antibodies attach to the same part of the same target and this decreases the potential for non-specific targeting. However, the strict specificity of these antibodies also decreases the probability that the antibody will bind to the target. You may wonder why the binding probability decreases. It is because 1. There is less potential target to bind to, and 2. There isn't a guarantee that every molecule of the target will be accessible to the antibody due to the unpredictable effects of fixation on tissue.

5. Let's say we are looking at a company's product page for an antibody we want to use. What information should be on the page to help inform us about whether the antibody will or will not work for our specific lab's goals? For example, here is a typical antibody product page: https://www.ptglab.com/products/ACHE-Antibody-17975-1-AP.htm
    a. Target species (a.k.a. Species reactivity)
    b. Host species
    c. Polyclonal or monoclonal
    d. Immunogen or target sequence
    e. Recommended applications (IHC, WB, IP, ELISA)
    f. Pictures of the antibody staining - not just western blots!
    g. The pictures show staining in the tissue type you plan to use
    h. Concentration of the product
    i. Storage buffer or additives (typically PBS w/ 0.02% azide)
    j. Reference info for publications that had used this antibody successfully (and specifically for your intended application, such as IHC in my case).
6. If a vendor states that an antibody is approved for use in IHC but does not provide a photo of IHC staining, should you trust their recommendation?
    a. Nope! This isn't to say that the vendor is lying, but it also suggests that the vendor hasn't tested the antibody with IHC. A student and I had been deceived by this situation for an antibody we ordered, and from now on I require pictures of stained tissue for any new antibodies I order. Sometimes I go a bit further, especially if an antibody product is expensive, and I see if there are publications that used the antibody. I then find those publications and look for pictures of what their staining looks like, assuming they performed IHC.
    b. Also, when I mention the term "a photo of IHC staining", I exclude western blot pictures. Since my focus is IHC, I want to see pictures of stained tissue. The conditions used for western blot staining are not the same as staining in tissue. This means that a picture of successful western blot staining with that antibody will tell you nothing about how well it can stain tissue.
7. Do you know the difference between cell culture photos versus tissue section photos on a vendor website?
    a. This question is more up to your own background knowledge. What I'd like to add though is that pictures can sometimes be ambiguous. For example, I had seen a picture of a dense cluster of BDNF-positive cells (BDNF stands for brain derived neurotrophic factor). In that picture, the staining was contained within the cells. https://www.sigmaaldrich.com/US/en/product/sigma/sab5700726
    [show picture from that product page]
    This gave me the impression that brain cells would also have BDNF confined to the cell bodies. After failing to visualize individual neurons after attempting to stain with an anti-BDNF antibody, I remembered where BDNF should be - mostly outside of neurons, as it is a form of communication they use. I checked the vendor page again, and saw

that the picture was of cancer cells that were modified to overexpress BDNF in vitro. This mistake was definitely not the vendor's fault - it was mine, but it stands as an example of how you should find pictures of staining with your exact tissue type or application if possible.

8. Is your target localized to specific cell compartments, or not?
    a. This is another general question where the answer probably differs for each of you. It is important to do your background literature research to figure out what the typical staining of your target looks like. If most stains are intracellular, then you should expect your stains to look the same. However, if the target molecule is more often found extracellularly and isn't anchored to anything - like the BDNF that I mentioned before - then you may not find pictures of cellular staining in the research literature, and you likely won't see it stained on tissue sections.
    b. For neurons, there is another tricky case. Certain neurotransmitters and peptides are not found in the cell body, but are instead mainly localized along the neuron's long axons or even at their terminals. What this means is that the target's staining could end up millimeters or even centimeters away from the originating cell body. There are some solutions to this issue, but those solutions are pretty complicated to execute. The easiest solution I recommend is to use in situ hybridization for the mRNA involved in the creation of those neurotransmitters or peptides. Generally, such mRNA sticks around the cell body more often, and can thus be connected to a cell more easily.
    https://www.novusbio.com/products/leu-enkephalin-antibody_nbp1-78328
    [show example of axonal staining for leu-enkephalin]
    c. In spite of the issue I just mentioned, there are some neurotransmitter-synthesizing enzymes that do stay mostly within the cell body. One example is GAD67, the enzyme that makes the neurotransmitter GABA. Staining for GAD67 will highlight neurons. In contrast, staining for GABA will probably highlight large hazy extents of brain areas. So, I recommend looking into what staining of enzymes looks like, in case you suspect that visualizing the product of that enzyme might be too difficult.
    [show example of GAD67 staining vs GABA staining]
9. Should I perform antigen retrieval if the vendor suggests it?
    a. Ehhhhhhh maybe. But I suggest not performing antigen retrieval, at least not in your initial tests of the antibody. Antigen retrieval is standard to some professions and certain ways of preparing tissue. I often see it applied to paraffin-embedded tissue. However, antigen retrieval can be harsher on tissue that is not paraffin-embedded. I also don't recommend performing it on free-floating tissue. Lastly, the boiling buffer variants of retrieval can be tricky to set up so that they are effective AND safe. With all of that said however, if you find that an antibody does not work in normal applications, it is worth at least giving antigen retrieval a try if you have time and the resources to do it.
10. If a company that we buy antibodies from suggests that we use a 1:100 dilution (stock to diluent), should we trust that suggestion? Why or why not?
    a. I say no. I don't mean to instill paranoia or imply that the vendor is lying. However, you cannot guarantee that their setup for testing the antibody will be the exact same as your setup. Thus, you *have* to do a dilution test on your own antibody. The vendors may even say on their page that "optimal dilutions should be determined by the user". I have an explanation of how to set up a dilution curve test in my free IHC lab manual, which you can find via my website - see the link in the description.

    b. Putting aside the need to do your own testing, it is still possible that some vendors are misleading. They may advise you use higher concentrations of the antibody in solution that what you actually need. This causes you to deplete your antibody stock sooner, and then you have to buy more… which you might imagine the seller is all for.
11. When a secondary antibody product is listed as “cross-adsorbed”, what might that mean, and why is it important?
    a. Antibodies that are cross-adsorbed might also say that they have minimal cross-species reactivity. This situation is important for multiple labeling. Let me define what multiple labeling means, then. It is when you have two different targets in the same tissue, and you want to label both of them with different colors. An example of multiple labeling would be an IHC procedure that eventually labels dopamine 1 receptors in green and GABA B receptors in red. Let’s say that in that case, the dopamine receptor antibody comes from mouse, and the GABA B receptor antibody comes from rabbit. Two secondary antibodies can be used to target those primaries: a donkey anti-mouse antibody, and a donkey anti-rabbit antibody. Lastly, each of these secondary antibodies would have different color fluorescent tags attached. But considering all of that, what if the donkey anti-rabbit antibody also sometimes binds to mouse primary antibodies? This would result in dopamine receptors being falsely labeled as GABA B receptors. Recall what I mentioned about antibodies sometimes working against the same target protein in other species. This rule applies for secondary antibodies that target the same general piece of primary antibodies.
       [showing diagram would be ideal]
    b. The process of cross-adsorption screens a secondary antibody so that all the antibody molecules that react to the wrong species are taken out, and only the antibody molecules that react to the correct species remain. It is important to look at the list of species that the vendor’s secondary antibody should not bind to. Here is one example for Jackson Immunoresearch
       https://www.jacksonimmuno.com/catalog/products/711-165-152
       [show webpage above]
12. Is there a collective database that has info on most antibody products available, rather than just doing normal web searches?
    a. Yes! There are a few. The one I have used most often is the Antibody Registry.
       https://antibodyregistry.org/
       [show webpage]
       Let’s take it for a spin. If I search for the target “dopamine 1 receptor”, we are given a lengthy list. The search isn’t perfect since I typed in the number 1 instead of the letter-number combo D1. However, we’re immediately given a list of various antibody products, vendors, and most importantly the publications these products have been used in. Something to be aware of is that not all of these antibody products are still being sold, but the information you find here can help point you to other vendors that might have what you are looking for.
13. Where can one find cheaper antibodies?
    a. Figuring out the answer to this question is of much interest to researchers that don’t have money piled up in cabinets. Sometimes there are non-profit repositories that maintain antibody stocks and cell lines that produce antibodies. These non-profit organizations are government-funded, and they charge far less for researchers working at non-profit institutions, such as most colleges and universities. The two organizations I

know of are the Developmental Studies Hybridoma Bank, also called DSHB for short, and the UC Davis NeuroMAb organization. Here are their sites, and I've put links in the video description.
https://neuromab.ucdavis.edu/
https://dshb.biology.uiowa.edu/
[showcase the two websites, and put the links in the video description]
You may be wondering, what is a low cost for an antibody product? Well, DSHB sells tissue culture supernatant for $45 dollars. Although this solution is somewhat diluted, the total amount of antibody molecules you get is still roughly equivalent to what commercial vendors would sell. So, you are getting the same amount of product but for one tenth the cost!

b. Interestingly, in the last five years or so, DSHB has begun distributing some of NeuroMAb's antibodies. This is good, as NeuroMAb themselves no longer directly distribute their own antibodies. At some point in the last decade, they had partnered with some companies, and some of those companies took their cheap antibodies and sold them at normal, several-hundred dollar antibody costs. I find that particularly egregious since the distributors are not even putting in the effort, costs, or resources for making the antibodies themselves, yet they are charging such a large premium on the products. To be clear, I don't blame NeuroMAb for this. I'm not sure what happened such that they couldn't distribute their own products any longer, but I imagine they did not have much choice.
c. Let me know if you are aware of other non-profit organizations that make and distribute antibodies. Post your findings in the comments!
d. If non-profit organizations do not have the antibody you need, you can inquire with specific vendors about whether they distribute samples of specific antibodies, or if they have smaller sizes for sale. The one I showed earlier has a smaller volume for purchase: https://www.ptglab.com/products/ACHE-Antibody-17975-1-AP.htm

Hopefully these ideas have helped you decide how to obtain the right antibodies for your research projects. Keep in mind that although following my tips will increase your chances of the IHC stains working out, you shouldn't expect a 100% success rate if you do a lot of different IHC stains. That said, the success rate should be greater than 80%. You should also keep in mind that there are other factors that could affect antibody staining. A prominent one is if the tissue is overfixed, meaning that it was kept in formaldehyde for too long. This will mask the targets of most antibodies and give a false negative. There are other reasons why an antibody product might not work that I won't get into here. Regardless, your success will be greater if you are careful in how you choose your antibodies from the vendors you browse on the internet. See you next video!